Serologic Responses to Eastern and Western Equine Encephalomyelitis Vaccination in Previously Vaccinated Horses*

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\section*{ABSTRACT}
A prospective study was performed to determine the serologic response of previously vaccinated horses to revaccination against eastern and western equine encephalomyelitis (EEE and WEE). Horses responded variably to each antigen, and some horses had low or undetectable antibodies 6 months after vaccination. Some horses did not develop increasing titers to EEE or WEE despite recent vaccination. Geometric mean titers peaked 2 weeks after revaccination and were significantly increased from before revaccination. Except for one horse, EEE:WEE titer ratios ranged from 0.25 to 2.0. Regular vaccination against EEE and WEE did not interfere with testing for Saint Louis encephalitis.

\section*{INTRODUCTION}
Eastern and western equine encephalomyelitis (EEE and WEE) and West Nile virus encephalomyelitis (WNV) are viral etiologies of equine neurologic disease. In the eastern United States, EEE is an important diagnostic differential for neurologic disease in horses. Mortality rates as high as 96% have been reported for horses affected with EEE.\textsuperscript{1} Horses that survive EEE may take months to improve and often have permanent neurologic deficits.\textsuperscript{2,3}

EEE virus cycles naturally between sylvatic hosts, such as wild birds, and mosquitoes. The mosquito species that maintain the virus in the sylvatic cycle (\textit{Culiseta melanura}) generally prefer to feed on birds and live in freshwater-forested swamps. Other species of mosquitoes that feed on either avian or mammalian hosts (\textit{Aedes} spp and \textit{Coquilletidia perturbans}) are

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more important for transmission of the virus to horses and humans. Human and equine cases of EEE tend to occur during or just following mosquito season in endemic areas.  

EEE, WEE, and WNV are all arboviruses, but EEE and WEE are alphaviruses and WNV is a flavivirus. Antigenic differences between these viral families prevent cross-protection for WNV by vaccination against EEE or WEE. Preventive measures against EEE, WEE, and WNV include regular vaccination and mosquito control.

Definitive diagnosis of EEE can be made postmortem with brain tissue using virus isolation or immunohistochemistry techniques. A difference of eightfold or greater between EEE and WEE titers has been reported to be diagnostic of EEE virus infection, regardless of vaccination status. In vaccinated horses, titers against EEE and WEE are higher and the ratio of EEE:WEE titer is lower (closer to 1) than in nonvaccinated horses. Comparison of paired acute and convalescent titers often is not possible because most horses affected with EEE die or are euthanized soon after the onset of clinical disease. A presumptive diagnosis of EEE or WEE may sometimes be made using a single serum sample if the titer is extremely high, but a peak titer may occur before the onset of clinical signs of neurologic disease. The serologic diagnosis of EEE and WEE is often confounded because many horses have been previously vaccinated and the interpretation of a single titer may be difficult. WNV cross-reacts in many serologic tests with other flaviviruses of the Japanese encephalitis serocomplex, including Saint Louis encephalitis (SLE). Antibody testing for antibodies against EEE, WEE, and SLE was performed using hemagglutination inhibition (HI). Sera were serially diluted in twofold increments, beginning at a 1:20 dilution. Antibody titers were determined as the highest serum dilution that inhibited agglutination of virus-adsorbed erythrocytes. Sera that had only a trace amount of HI antibody were read and recorded as less than 1:20.

Statistical Analysis
If the titer for a horse was negative or the inverse titer was less than 20 to either virus, the EEE:WEE ratio was not calculated and a value of 0 was used to calculate the mean inverse titer. Data were analyzed using the general linear model (Proc GLM, SAS) for repeated measures analysis of variance on log transformed inverse
were significantly \( P = .02 \) increased from Time 0 values only at Week 2 (Figure 1). The geometric mean EEE titer peaked at Week 2.

The mean ± SD inverse WEE titer before vaccination was 35.00 ± 22.76 (range = 20 to 80). The mean inverse WEE titer peaked at Week 2 (63.33 ± 38.92; range = 20 to 160). The WEE titer also peaked at Week 2 in eight of 12 horses. Western equine encephalomyelitis titer was highest before vaccination in two horses, and titers remained unchanged for the duration of the study in two horses. One horse became EEE-seronegative 24 weeks after vaccination (Table 1). Geometric mean EEE titers were significantly \( P = .02 \) increased from Time 0 values only at Week 2 (Figure 1). The geometric mean EEE titer peaked at Week 2.

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DISCUSSION

Over 50% of horses diagnosed with EEE virus infection in one study had been vaccinated within 1 year from the onset of neurologic disease. The horses used in this study responded variably to vaccination against EEE and WEE antigens. Forty-two percent of the horses did not respond with an increasing titer to vaccination against EEE and 33% did not respond against WEE. Four horses had low or undetectable antibodies within 6 months after vaccination. Another vaccination study also reported that individual horses responded differently to vaccination against EEE and WEE. In that study, HI antibodies were not found in some horses as soon as 2 months after vaccination, and individual horses often did not maintain detectable titers against both EEE and WEE viruses concurrently. Antibody titers not differ from each other at any sampling time (Figure 1). After vaccination, titers to EEE did not increase for two horses, and another two horses did not have increased titers to WEE. However, in each case, the horse developed increasing antibody titer to the other virus in the vaccine, indicating a normal immune response.

With the exception of one horse, EEE:WEE ratios were within the range of 0.25 to 2.0 throughout the study (Figure 2). The mean EEE:WEE ratio was 1.64, and the median ratio was 1. One horse had an EEE:WEE ratio of 4 before vaccination that rose to 8 at Week 2. The EEE:WEE ratio for that horse was 16 at Week 4 after vaccination, declining to 8 at Week 12 after vaccination, where it remained for the duration of the study.

All horses remained seronegative for SLE for the duration of the study.

Figure 1. Geometric mean (± SD) inverse eastern and western equine encephalomyelitis (EEE and WEE) titers in horses vaccinated against EEE and WEE 6 months previously (Time 0 = revaccination). Asterisks indicate significant differences from titer before revaccination (P < 0.05).
produced by vaccination may overlap with titers caused by clinical EEE virus infection.9

The results of the present study support the conclusions of Brewer and Mayhew1 regarding the usefulness of a difference of eightfold or more in the EEE:WEE ratio for the diagnosis of EEE virus infection. However, the study demonstrated that it is possible for a clinically normal, recently vaccinated horse to have an EEE:WEE ratio of 8 or greater. With the exception of one horse, vaccinated horses had an EEE:WEE ratio of 2 or less. In a study of the efficacy of a trivalent inactivated equine encephalomyelitis vaccine, EEE:WEE ratios also did not exceed 2 following vaccination.8 One mare in the present study had an EEE:WEE ratio of 8 or greater at all times, except before vaccination. It is possible that the mare was naturally exposed to EEE virus soon after vaccination and responded with an increased titer.

Clinical EEE has also been associated with recent administration of an incompletely inactivated vaccine.10 The elevation in the EEE:WEE ratio in the mare in the present study can be explained in part by her differential response to vaccination against EEE and WEE antigens. The mare maintained an inverse WEE titer of 20 for the duration of the study, while her inverse titer against EEE increased up to fourfold from before vaccination. Detection of IgM antibodies against EEE virus is helpful to diagnose acute infections; however, IgM antibodies can be produced by recent vaccination9 and were not assayed in this study. Diagnostic use of the EEE:WEE ratio should be limited to horses with consistent clinical signs of EEE because the horses reported by Brewer and Mayhew1 had clinical signs of neu-

### TABLE 2. Inverse Western Equine Encephalomyelitis Titers for Horses Previously Vaccinated with a Multivalent Vaccine at 6-Month Intervals and Revaccinated (Time 0) to Determine Serologic Responses to Each Antigen

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Mean ± 35.00 ± 63.33 ± 41.67 ± 40.00 ± 46.67 ± 38.33 ± 41.67 ± 41.67 ±

SD 22.76 38.92 24.80 25.58 26.05 21.67 24.80 26.23
rologic disease and the horse in the present report with an EEE:WEE ratio greater than 8 remained asymptomatic.

No horses became seropositive for SLE, indicating that they were not exposed to flaviviruses such as SLE and WNV during the study. This indicates that regular vaccination against the alphaviruses EEE and WEE does not interfere with HI testing for SLE as a screening test for WNV exposure. However, more definitive serologic tests, such as IgM-capture ELISA, are likely to be more reliable for the diagnosis of WNV.

Vaccination against EEE and WEE at least every 6 months appears to be a reasonable practice in endemic areas where mosquito populations may persist for most of the year. Horses are apparently susceptible to EEE virus infection within 1 year after vaccination. This study and others have shown that horses respond variably to vaccination against EEE and WEE viruses and that some horses may become seronegative to either virus within 6 months of vaccination or fail to maintain detectable titers against both viruses concurrently. Some horses in the present study did not develop increasing titers to EEE or WEE despite recent vaccination. A protective vaccination titer against EEE or WEE is unknown. However, Barber et al reported that seronegative horses remained refractory to challenge with EEE or WEE virus 3, 8, and 12 months after vaccination. The use of serology alone as a method to determine optimal revaccination intervals or to indicate if vaccination against EEE or WEE is necessary should be discouraged.

REFERENCES


